Not only the bone marrow and spleen, but also the liver, an organ with a cell composition in newborn rats similar to that of the bone marrow [4], also reacted to hormone administration. Under the influence of the hormones the number of nucleated cells in the liver was reduced, possibly reflecting the decline of hematopoiesis in that organ.

An external factor such as hormones thus caused acceleration of functional maturation of the blood system, in the form of activation of bone marrow function and inhibition of hematopoiesis in the liver.

The responses observed point to the ability of the fetal hematopoietic apparatus to react to an external environmental factor such as sex hormones.

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SYNTHESIS AND LOCALIZATION OF α-FETOPROTEIN DURING REGENERATION OF THE LIVER IN MICE

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The main site of localization of α-fetoprotein (αFP) in mouse liver regenerating after CCl_4 poisoning or partial hepatectomy was in the typical mature hepatocytes that account for not more than a few per cent of the total number of residual hepatocytes. Morphologically they were indistinguishable from the main mass of hepatocytes and they retained on their surface bile capillary antigen. The change in their number and in the brightness of their fluorescence in liver sections corresponded to the dynamics of the αFP level in the animals' serum. During regeneration of the liver in mice αFP is evidently produced mainly by mature hepatocytes.

KEY WORDS: α-fetoprotein; localization in sections; immunofluorescence; hepatocytes; regeneration of the liver.

The appearance of the embryo-specific protein known as α-fetoprotein (αFP) in adult mice after partial hepatectomy was described by Abelev et al. [1]. In mice and rats after hepatectomy or CCl_4 poisoning the blood αFP level as a rule rises sharply on the second day, reaches a maximum on the third to fourth day, and then falls by the seventh to tenth day to its original values [1, 2, 4, 8]. The nature of the cells which synthesize αFP

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TABLE 1. αFP in Serum and Liver Sections of Mice Poisoned with CCl_4 Vapor

Time (in hours) after poisoning	Number of mice	Serum αFP concentration*, μg/ml	Immunofluorescence picture in sections
0	20	0.4 (0.1-0.9)	No αFP found
18	6	1.2 (0.6-2.6)	Centrolobular necrotic foci present; damaged hepatocytes do not yet contain γ-globulin; no cells with αFP found
24	16	1.2 (0.3-3.2)	Extensive centrolobular necrotic foci with bright fluorescence of γ-globulin; two hepatocytes with αFP were found on boundary with necrotic foci in a mouse whose serum contained αFP in concentration of 1.6 μg/ml
30	6	3.5 (0.8-6.7)	Extensive areas of necrosis bounded by a few pale hepatocytes with αFP
42	3	19.3 (10-29)	Extensive areas of necrosis; number and intensity of fluorescence of hepatocytes with αFP increased
48	9	72 (2.8-190)	Area of necrotic foci reduced; number and intensity of fluorescence of hepatocytes with αFP
72	9	180 (35-350	Maximal number and intensiy of fluorescence of hepatoxytes with αFP
96	6	120 (20-325)	Number of hepatocytes with αFP appreciably reduced; necrotic foci have disappeared
120	6	50 (15-90)	Hepatocytes with αFP remain in not all mice; weak fluorescence of epithelium of bile ducts and bright, of solitary small cells
144	2	20	No cells with αFP found
192	1	0.5	No cells with αFP found

*Serum αFP concentration determined by EPAG method and by radial immunodiffusion using the same standard αFP. Mean values and maxima and minima shown for each time after CCl_4 poisoning.

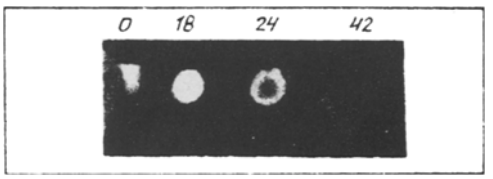

Fig. 1. Dynamics of serum αFP concentration in mice after poisoning with CCl_4 vapor. All samples of serum diluted 1:8. Dilution of antiserum against αFP in polyacrylamide gel 1:1200. 0, 18, 24, 42) Hours after poisoning animal.

in the adult organism during regeneration of the liver, in the acute phase of clinical carcinogenesis, and during tumor formation in the liver has been discussed in the literature. It has been suggested that αFP is synthesized by differentiated hepatocytes, by a certain population of these hepatocytes only, and, finally, by immature, transitional forms of hepatocytes [2].

The object of the investigation described below was to discover what cells can produce αFP in the regenerating mouse liver by comparing the picture of αFP fluorescence in liver sections with the dynamics of its serum level in the same animals. To obtain further information on the cells a specific hepatocyte marker was used: an antigen present in high concentrations in those parts of their surface which form bile capillaries (BCA) [10].

EXPERIMENTAL METHOD

Regeneration of the liver after poisoning by CCl_4 vapor [4] was studied in male SWR mice aged 3-4 months. Partial hepatectomy [12] was performed on male C3HA mice of the same age. At various times after these procedures blood samples were taken from the retroorbital sinus of the mice for determination of the serum αFP concentration by precipitation in agar with a standard test system [9], by radial immunodiffusion [13], or by combined electrophoresis and precipitation in polyacrylamide gel (EPAG) [3] (Fig. 1).

The localization of αFP, BCA, and mouse γ-globulin (MGG) was studied in paraffin sections (3 μ) through the liver by the indirect immunofluorescence method [16]. Pieces of tissue were fixed with a mixture of ethanol and acetic acid or acetone at 4°C. The technique of processing the material was fully described previously [11].

The preparation of mouse αFP, purified by electrophoresis in polyacrylamide gel, and the antiserum against it [16] were obtained from A. K. Yazova. The methods of isolating monospecific antibodies against αFP and verifying their specificity before neutralization with the αFP preparation were described previously [11]. Monospecific antibodies against BCA were obtained from N. I. Khramkova and T. D. Beloshapkina [10]. Monospecific antiserum against MGG was obtained by immunizing rabbits by injecting MGG antigen into their lymph nodes (O. M. Lezhneva).

The protein concentration in the αFP preparation, dialyzed against distilled water, was determined spectrophotometrically, taking the value of $E^{1\%}_{1cm}$ at 278 nm for mouse αFP to be 4.15 [15].

EXPERIMENTAL RESULTS

Changes in the mouse liver after intraperitoneal injection of small doses of CCl_4 described in the literature [14] were observed under the present experimental conditions also. Between 18 and 24 h after poisoning the hepatocytes in the center of the lobule died and up to 50% of the lobule was affected by necrosis. Hepatocytes adjacent to the foci of necrosis formed a special zone: They differed from the remainder of the parenchyma by containing fewer vacuoles and having increased basophilia of their cytoplasm. By the third to fourth day the foci of necrosis were invaded by connective tissue and by the fifth to sixth day they had virtually disappeared.

Cells containing αFP were first found 24 h after CCl_4 poisoning in liver sections from a mouse in whose blood the αFP level was raised (Table 1). These were solitary hepatocytes with weak fluorescence located on the boundary with necrotic foci. On the second day in most mice the number of hepatocytes containing αFP and the intensity of their fluorescence increased sharply (Fig. 2a, b, g, h). The brighest fluorescence and the largest number of hepatocytes with αFP were usually observed on the third day, but at that time their number did not exceed 5-7% of the total number of hepatocytes (Fig. 2c, d). They were evidently not uniformly distributed in the liver tissue: Their number and the intensity of their fluorescence varied in sections from different parts of the same piece and from different pieces of liver of the same animal. On the whole, however, examination of several liver sections from each mouse during the first 3 days after poisoning showed in most cases a pattern of fluorescence which corresponded to the αFP concentration in the blood of the same animal. Since αFP is present in the blood of animals with a regenerating liver, it had to be established whether its discovery in the cells was due to their penetration by serum as a result of injury to their membranes. For this purpose, simultaneously with αFP, the localization of MGG as a serum marker was determined in neighboring sections [11]. By using sections 3μ thick it was possible to identify the same hepatocyte in three or four successive sections. The overwhelming majority of hepatocytes with αFP did not contain MGG and only those cells were counted (Fig. 2). The characteristic arrangement of hepatocytes with αFP was observed in the liver regenerating after CCl_4 poisoning. In most mice they were found only in the zone of cells directly adjacent to the necrotic foci (Fig. 2). These cells possibly had suffered sublethal damage of the paranecrosis type [7]. In some

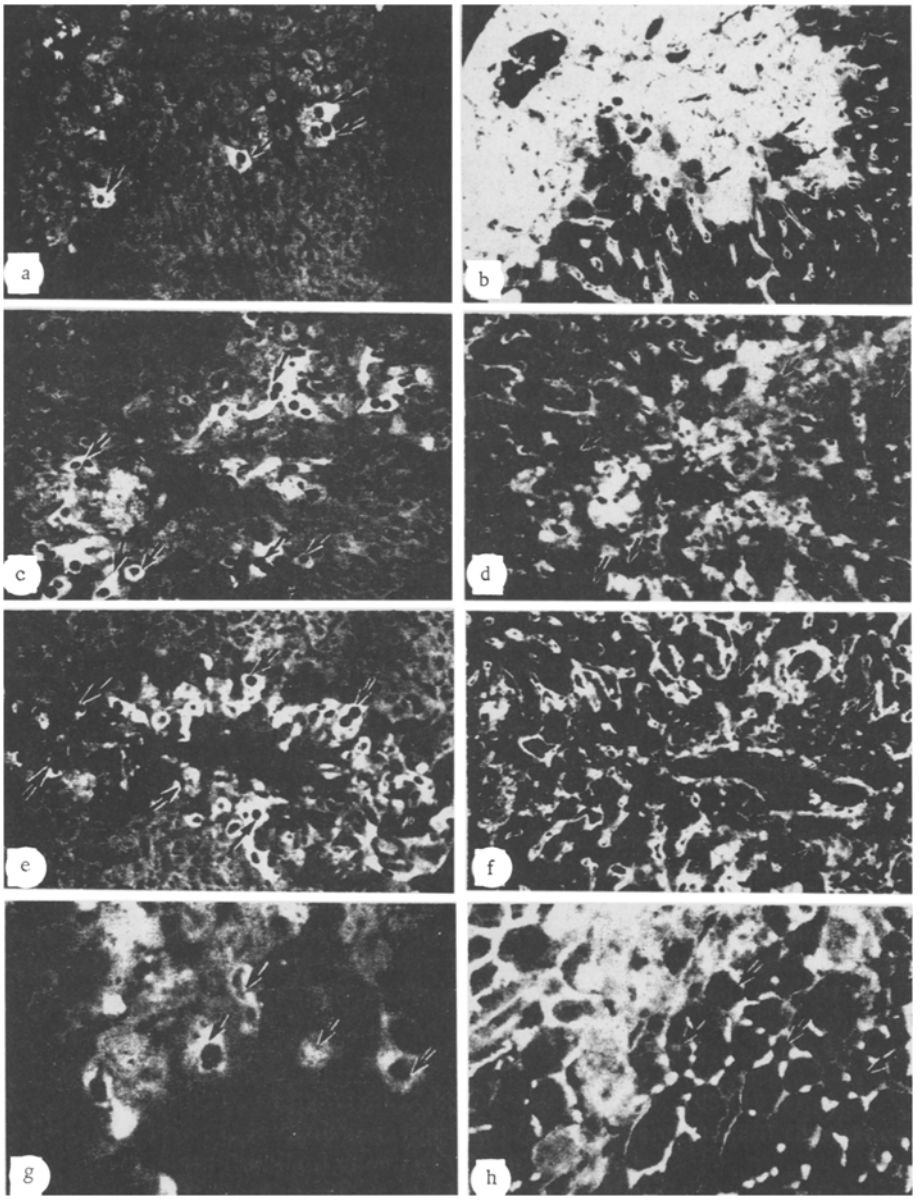

Fig. 2. αFP, MGG, and BCA in sections of mouse liver regenerating after CCl_4 poisoning: a, c, e, g) sections incubated with antibodies against αFP, b, d, f) with antibodies against MGG, h) with antibodies against BCA. Short arrows point to same hepatocytes in two neighboring sections. a, b, g, h) Liver sections 48 h, c, d) 72 h, and e, f) 96 h after CCl_4 poisoning. In fragment e the three long arrows on the left point to unidentified small cells containing αFP. Fixation with ethanol–acetic acid (a-f) and acetone (g, h); a, b, c, d, e, f) objective 20 ×, homal 3 ×; g, h) objective 40 ×, homal 3 ×.

mice they were found both in that zone and in the periportal region. In some animals hepatocytes with αFP were found in the periportal region only, close to the triads.

Judging from the size of the nuclei, hepatocytes with αFP belonged to different ploidy classes, and they included cells with both one and two nuclei. Exactly the same pattern of αFP localization also was obtained by the use of the other fixative, acetone. By fixation in this way the localization of αFP and BCA could be determined simultaneously. The results showed that on the first 3 days after poisoning hepatocytes with αFP contained this specific marker of mature hepatocytes on their surface (Fig 2). On the fourth day the number of cells containing αFP fell sharply, but there was a corresponding increase in the percentage of small cells, most of which

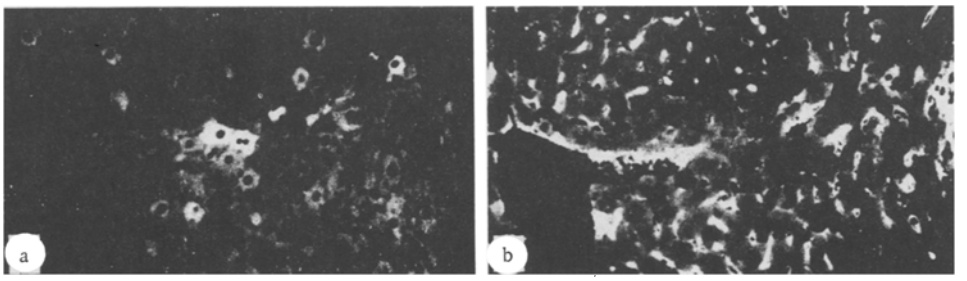

Fig. 3. αFP in sections of mouse liver regenerating after partial hepatectomy (48 h, fixation with ethanol–acetic acid): a) incubation with antibodies against αFP, b) with antibodies against MGG. Objective 20 ×, homal 3 ×.

evidently were small hepatocytes, although some could not be identified. On the fourth to sixth day proliferation of bile ducts was observed, and weak fluorescence of αFP was found in the cytoplasm of the epithelium lining some of them. By the sixth to seventh day reliable fluorescence of αFP in the hepatocytes had usually disappeared, weak fluorescence still remained in individual small cells and in parts of the blood vessels, and the blood αFP concentration was very low (Table 1).

During regeneration of the liver in mice after hepatectomy elevation of the blood αFP level was detected on the second day by precipitation in agar. The first cells with αFP were found after 48 h. These also were hepatocytes, usually not the largest, and some contained two nuclei (Fig. 3).

The largest number of hepatocytes with αFP was found on the third day in mice with a high blood αFP concentration, but in this case also their number did not exceed a few per cent of the total number of hepatocytes. On the second to third day after the operation the location of the hepatocytes with αFP in the lobule did not follow any clearly recognizable pattern. On the fourth day, despite the high blood αFP level, few hepatocytes with αFP still remained, and their fluorescence was weak, and mainly at the periphery of the lobules.

The main site of localization of αFP in the regenerating liver of mice both after heptectomy and after CCl_4 poisoning was thus in typical mono- and binuclear mature hepatocytes, which accounted for not more than a few per cent of the total number of hepatocytes still remaining. Morphologically they were indistinguishable from the main mass of hepatocytes and they preserved BCA on their surface. The change in number and brightness of the fluorescence of the hepatocytes with αFP in the liver sections during the first 3 days after poisoning corresponded to the dynamics of the blood αFP level in these animals, but starting from the fourth day the decrease in the number of hepatocytes containing αFP preceded the fall in its blood concentration. To determine whether hepatocytes with αFP arise as a result of proliferation of noncompetent precursors or whether they pre-exist in the liver of adult mice, experiments were carried out in which mice with a regenerating liver were given repeated injections of labeled thymidine. The results suggested that during regeneration of the liver in mice most αFP production takes place in ordinary mature hepatocytes. Other structures, in which they also were found, may possibly participate in αFP synthesis under these conditions also: small cells in the parenchyma and the epithelium of the bile ducts. It is unlikely, however, that their contribution is decisive under the experimental conditions used. Other experimental modes are required to analyze their role in αFP synthesis.

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EFFECT OF PRECEDING STRESS ON MITOTIC ACTIVITY OF THE REGENERATING LIVER

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For 2 h male rats were immobilized 14 h or 24 h before partial hepatectomy. Some of these animals received subcutaneous injections of theophylline immediately after immobilization and next day. Under the influence of stress a tendency was observed for mitotic activity of the regenerating liver to be increased. This effect was particularly marked in rats receiving theophylline additionally.
KEY WORDS: regeneration of the liver; mitotic activity of hepatocytes.

Numerous investigations have shown that stress leads to inhibition of mitotic cell activity. This has been found mainly by the use of objects such as the corneal epithelium. However, stress does not inhibit but increases the number of mitoses in the epithelial cells of the crypts of the small intestine [2, 3, 5]. It has also been found that the action of a stressor before partial hepatectomy increases DNA synthesis in the regenerating liver [8, 9].

The object of this investigation was to study the effect of stressor action preceding partial hepatectomy on mitotic activity of the regenerating liver and also to investigate the action of theophylline, an inhibitor of cyclic AMP phosphodiesterase, on the result of such stress.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male rats with a mean weight between 180 and 231 g. The animals as a whole were divided into five groups (Table 1). The animals of group 1 acted as the control and underwent partial hepatectomy with the removal of about 70% of the mass of the liver. The animals of the other groups were immobized 14 or 24 h before hepatectomy for 2 h by fixation to the bench. Some rats immediately after the end of immobilization and also next day (before resection of the liver) received subcutaneous injections of theophylline solution in a dose of 10 mg/100 g body weight. All the animals were killed 30 h after resection of the liver, which was always performed at 3-4 p.m. Pieces of liver were fixed in Carnoy's fluid and embedded in paraffin wax. Sections were stained by Feulgen's method. The total number of mitoses and the various phases of mitosis separately were counted in 100 fields of vision of the microscope (objective 40, ocular

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